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Estimation of total phenolic acids, flavonoid compounds and antioxidant activity of *Ficus deltoidea* varieties and their HPLC profiles with different solvents

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ABSTRACT

The aim of this study was to evaluate the effect of methanol and ethanol extraction on antioxidant activities, total polyphenol, phenolic acid and flavonoid content of different *Ficus deltoidea* varieties. Our findings revealed that fresh leaves of *F. deltoidea* var. *kunstleri*; FDK1 had the highest total polyphenol, phenolic acid, flavonoid and antioxidant activities compared to that of other varieties. Ethanol extraction of FDK1 showed the highest activity in total antioxidant (DPPH) (4.48 mg TE/g FW), polyphenol (1.13 mg GAE/g FW) and flavonoid content (6.81 mg RE/g FW) while methanol extraction showed the highest activity in total antioxidant (FRAP) (2.43 mg TE/g FW) and phenolic acid content (4.54 mg GAE/g FW). HPLC quantification in dried leaves of FDK1 found out rutin exhibited higher than naringin. The highest rutin and naringin was found in FDK1 and FDT2 (12.83 and 3.04 μg/g DW). These results demonstrate that extraction solvent and *F. deltoidea* variety influence the activity of total antioxidant, polyphenol, phenolic acid and flavonoid content.

Keywords: Antioxidant, *Ficus deltoidea*, flavonoid, phenolic acid, polyphenol

ABSTRAK

Tujuan kajian ini adalah untuk menilai kesan pengekstrakan metanol dan etanol ke atas aktiviti antioksidan, jumlah polifenol, asid fenolik dan kandungan flavonoid dari pelbagai varieti *Ficus deltoidea*. Hasil mendapati daun segar *F. deltoidea* var. *kunstleri*; FDK1 mempunyai jumlah polifenol, asid fenolik, flavonoid dan antioksidan tertinggi berbanding dengan varieti lain. Ekstrak etanol FDK1 menunjukkan aktiviti tertinggi dalam antioksida keseluruhan (DPPH) (4.48 mg TE/g FW), polifenol (1.13 mg GAE/g FW) dan kandungan flavonoid (6.81 mg RE/g FW) manakala pengekstrakan metanol menunjukkan aktiviti tertinggi dalam antioksidan keseluruhan (FRAP) (2.43 mg TE/g FW) dan kandungan asid fenolik (4.54 mg GAE/g FW). Kuantifikasi HPLC dalam daun kering FDK1 mendapati kandungan rutin lebih tinggi daripada naringin. Rutin dan naringin tertinggi didapati dalam FDK1 dan FDT2 (12.83 dan 3.04 μg/g DW). Keputusan ini menunjukkan bahawa pelarut pengekstrakan dan varieti *F. deltoidea* mempengaruhi aktiviti antioksidan keseluruhan, polifenol, asid fenolik dan kandungan flavonoid.

Kata Kunci: Antioksidan, asid fenolik, *Ficus deltoidea*, flavonoid, polifenol

INTRODUCTION

Ficus deltoidea (*F. deltoidea*) has been recognized for many generations for its health benefits and medicinal properties. It is a native to Southeast Asia especially Malaysia, Philippines and Indonesia. The leaf of *F. deltoidea* contains distinct characteristics of human reproductive organs thus, it is used particularly for female and male fertility treatments (Mat *et al*., 2012). Medicinal values of *F. deltoidea* include facilitating childbirth, postpartum medication and to contract cervix muscles for female, provides extra energy, regulates blood system, headache, toothache, cold, wound, sores and rheumatism (Sulaiman *et al*., 2008). Apart from that, traditional usage of *F. deltoidea* extract includes reducing sugar level in blood, decreasing blood pressure, cholesterol and lipids, treatment for migraine, nausea, joints and piles pain, delaying menopause, toxin removal from the body and reducing the risk of cancer (Aris *et al*., 2009).

Plant species with antioxidant compounds are potent scavengers of free radicals and useful in the prevention of arteriosclerosis, cancer, diabetes, neurodegenerative diseases and arthritis. Phytochemicals exerting antioxidant actions are largely being recognized as beneficial to human health and disease prevention by interfering in the processes involved in reactive oxygen species (ROS) mediated pathologies, e.g. coronary diseases, cancer, age-related degenerative brain disorders and infectious diseases (Soobrattee *et al*., 2008). ROS or free radicals are chemical species which contain one or more unpaired electrons due to which they are really unstable and cause damage to other molecules by extracting electrons from them, in order to attain stability (Ali *et al*., 2005). They have strong tendencies to impair the proper functioning of the immune system, which leads to infection and

degenerative diseases. Under normal condition, the level of ROS in the cell is in equilibrium (Apel and Hirt, 2004). Due to biochemical processes occurring in the body, it is normal for ROS to be present regularly in the body but, the danger begins when the ROS increase to an abnormal level (Vankar *et al.*, 2006).

Phytochemicals such as phenolic compounds, including phenolic acids and flavonoids are bioactive components in plants which known for its free radical scavenging activities (Sepahpour *et al.*, 2018). Different type, number and position of functional groups of bioactive compounds resulting in variations in chemical properties which can influence the solubility of these compounds in different solutions or solvents (Meneses *et al.*, 2013). Other than that, concentration of extracted compounds significantly influenced by the solvent used during extraction due to different polarities of the compounds (Norra *et al.*, 2016). Solvent such as water (aqueous), methanol, ethanol and acetone are the most commonly used solvents for extracting bioactive compounds from fruits, vegetables, and other food products. Therefore, the overall objective of this study was to investigate the antioxidant activity, total polyphenol, phenolic acid, flavonoid and flavonoid compounds in methanol and ethanol extract from different *F. deltoidea* varieties.

MATERIAL AND METHODS

Plant Materials

A total of six accessions of *F. deltoidea* plants were purchased from the Department of Agriculture in Serdang, Malaysia, grown and maintained in the glasshouse of Universiti Putra Malaysia in natural environmental conditions with temperature 35/25 °C and a photoperiod of 16 hrs light/8 hrs dark according to temperature and day length during day/night. The plants were grouped into their respective variety by studying the leaf shape and morphology (Corner, 1969). Two accessions per variety were chosen for this study; var. *kunstleri* (FDK1 and FDK2), var. *trengganuensis* (FDT1 and FDT2), and var. *angustifolia* (FDA1 and FDA2). Leaf explants were used in this study because they were easily available compared to other plant parts. Prior to experiments, the leaves were cleaned under running tap water before being divided into two parts. The first part was dried in an oven at 50 °C for two days, or until the weight was constant for flavonoid separation using HPLC. The second part of the leaf explants was freshly used for determination of total antioxidant and phenolic compounds. All *F. deltoidea* varieties used in this study were deposited at the herbarium of Biodiversity Unit, Institute of BioScience, Universiti Putra Malaysia with voucher numbers as follows; FDK1=SS2257/13, FDK2=SS2258/13, FDT1=SS2265/13, FDT2=SS2266/13, FDA1=SS2262/13 and FDA2=SS2263/13.

Chemicals and Reagents

2,2-diphenyl-1-picrylhydrazil (DPPH), 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ), Trolox, Folin-Ciocalteu's phenol reagent and catechin were purchased from Sigma Co. St. Louis, Missouri, USA. Methanol, ethanol, sodium nitrite, acetic acid, sodium hydroxide, aluminium chloride, gallic acid, iron (lll) chloride hexahydrate and sodium carbonate were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

Preparation of Extracts for Total Antioxidants and Total Polyphenol Content

The extraction was conducted according to the method modified from Wong *et al.* (2006). A total of 0.5 g fresh leaf of each of the six accessions was cut into small pieces and placed in a 150 ml conical flask. Methanol and ethanol extracts were obtained by adding 25 ml of methanol or ethanol in flasks covered with aluminium foil. The homogenate was later maintained on an orbital shaker at room temperature for an hour. The samples were filtered using Whatman No. 1 filter paper and the extracts were stored at -80 °C freezer.

Preparation of Extracts for Total Phenolic Acids and Total Flavonoid Content

The extraction was conducted using a modified method by Marinova *et al.* (2005). *F. deltoidea* leaves weighing 0.5 g was ground using pestle and mortar in 50 mL of methanol or ethanol. Both extracts were centrifuged for 5 mins at 14000 rpm. Supernatants were collected and kept in -80 °C freezer before use.

Determination of Total Antioxidant Content

DPPH Free Radical Scavenging Assay

The assay was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH), employing the method described by Wong *et al.* (2006). Initial absorbance of DPPH in methanolic solution was measured at 515 nm using spectrophotometer (UV-2602, Labomed, Inc. USA) for control treatment. A total of 40 µl of the extract was added to 3 ml of 0.1 mM methanolic DPPH solution. The mixture was incubated at room temperature for 30 mins before changes in absorbance value at 515 nm was measured. The total antioxidant content was expressed as mg Trolox equivalent per gram fresh weight (TE/g FW) of leaf sample.

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was conducted according to Benzie and Strain (1996). Two hundred microlitre of the extract was added with 3 ml of FRAP reagent, prepared with a mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20 mM $FeCl.6H_2O$ at the ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37 °C for 30 mins. The increase in absorbance was measured using spectrophotometer at 593 nm. The total antioxidant content was expressed as mg Trolox equivalent per gram fresh weight (TE/g FW) of leaf sample.

Determination of Phenolic Compounds

Total Polyphenol Content

A total of 100 µl of extract was added with 2.5 ml of Folin-Ciocalteu reagent which was diluted 10 times. After 5 mins of reaction, 2.5 ml of 7% of sodium carbonate was added. The mixture was incubated at room temperature for an hour before the absorbance at 725 nm was measured. Total polyphenol content was expressed as mg gallic acid equivalent per gram fresh weight (GAE/g FW).

Total Phenolic Acid Assay

The assay was conducted using Folin-Ciocalteu method as described by Singleton and Rossi (1965). One milliliter of the extract was added to a flask containing 9 ml of distilled water. One ml of Folin-Ciocalteu's phenol reagent was added and the mixture was thoroughly mixed. After 5 mins, 10 ml of 7% Na_2CO_3 was added. The mixture was diluted to 25 ml by adding 4 ml of distilled water, and was incubated at room temperature for 90 mins. The absorbance was then measured using spectrophotometer at 750 nm. The total phenolic acid content was expressed as mg gallic acid equivalent per gram fresh weight (GAE/g FW).

Total Flavonoid Assay

Total flavonoid assay was conducted according to Marinova *et al.* (2005) using aluminium chloride colorimetric method. One milliliter of the extract was added with 4 ml of distilled water in a flask. After that, 0.3 ml of 5% $NaNO_2$ was added. After 5 mins, 0.3 ml of 10% $AlCl_3$ was added. After the sixth minute, 2 ml of 1 M NaOH was added. Then the mixture was made to 10 ml by adding 2.4 ml distilled water. The mixture was mixed and the absorbance later measured at 510 nm. Total flavonoids content was expressed as mg rutin equivalent per gram fresh weight (RE/g FW).

Preparation of Leaf Extract and Standard for Flavonoid Separation using HPLC

The extraction for flavonoid content using HPLC was carried out by a hydrolysis method (Crozier *et al.*, 1997). A total of 0.25 g dried sample was extracted with 10 mL of 60% of methanol in aqueous containing 20 mM sodium diethyldithiocarbamate (NaEDTC) as an antioxidant. The mixture was added with 2.5 mL of 6 M HCl and transferred to a round bottom flask. The hydrolysis process started when the mixtures were refluxed at 90 °C for 2 hrs. After the reflux process, the extracts were cooled to room temperature before filtered through 0.45 µm filter (Minisart RC 15, Sartorius, Germany).

Naringin and rutin were prepared by weighing 1 mg each and dissolved in 1 mL of methanol. The dissolved standard compounds were then filtered through 0.45 µm filter (Minisart RC 15, Sartorius, Germany). Different concentrations of standard compounds were prepared to construct a standard curve.

The leaf extracts and flavonoid standards were analyzed using reverse-phase high-performance liquid chromatography (HPLC) from the Waters (Milford, MA, USA). Reverse-phase separations were carried out at ambient temperature using 150 x 3.9 mm I.D., 4 µm C_{18} Nova-Pak column from Waters (Milford, MA, USA). The column was eluted at a flow rate of 1 ml/min in gradient mode, with acetonitrile in pump A and ultra-pure water, which was adjusted to pH 2.5 with trifluoroacetic acid (TFA) in pump B. The gradient of acetonitrile to water were analyzed in ratio of 1 to 35% at 365 nm for 30 min.

Statistical Analysis

The observations were replicated thrice for each experiment. All data were presented as mean ± standard deviation of three replicates using SAS software Least significant differences (LSD) test was used to evaluate the difference between treatment means at 95% confidence interval.

RESULTS AND DISCUSSION

Antioxidant Activities in Methanol and Ethanol Extracts

From Figure 1, total antioxidant was found higher in ethanol extracts compared to that of methanol extracts. Total antioxidant content of methanol extracts using DPPH method was ranged from 2.3 to 3.6 mg TE/g FW, while 2.4 to 4.48 TE/g FW for ethanol extracts. Leaf extract of FDK1 contained the highest total antioxidant content in both methanol and ethanol extracts with 3.60 and 4.48 mg TE/g FW, respectively. The lowest for both extracts can be detected in FDA1 leaf extract with 2.30 and 2.40 mg TE/g FW for methanol and ethanol extracts, respectively. Other extracts displaying high total antioxidant contents were FDT1, FDK2 and FDT2 for methanol extract (3.22, 3.16 and 3.09 mg TE/g FW) and FDT1, FDT2 and FDK2 for ethanol extract (3.70, 3.30 and 3.22 mg TE/g FW).

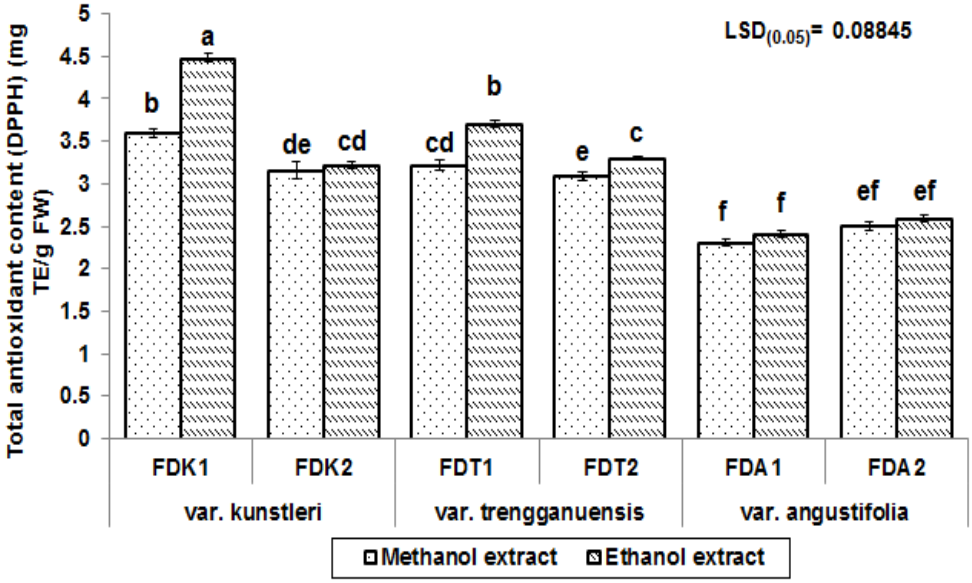

Figure 1: Total antioxidant content (DPPH method) of *Ficus deltoidea* varieties in methanol and ethanol extractions. Bar indicates the standard error of mean (n=3). Means with different letters are significantly different at $p<0.05$ from each other according to LSD post hoc analysis.

Figure 2 shows the total antioxidant content using FRAP method which was expressed as mg TE/g FW. All of *F. deltoidea* leaf extracts showed higher activity in ethanol extract compared to that of methanol extract, except for FDK1 leaf extract. Leaf extract of FDK1 showed slightly higher activity in methanol extract compared to that of ethanol extracts with 2.43 and 2.38 mg TE/g FW, respectively. The activity of total antioxidant in ethanol extract of FDK2 was considered high, followed by FDT2 and FDT1 lead extracts (2.00, 1.68 and 1.50 mg TE/g FW). The lowest total antioxidant activity for ethanol extract was found in leaf extract of FDA2 with 0.87 mg TE/g FW. Methanol extract showed lower activity of total antioxidant content compared to that of ethanol extract, with the lowest was found in FDA2 leaf extract (0.75 mg TE/g FW).

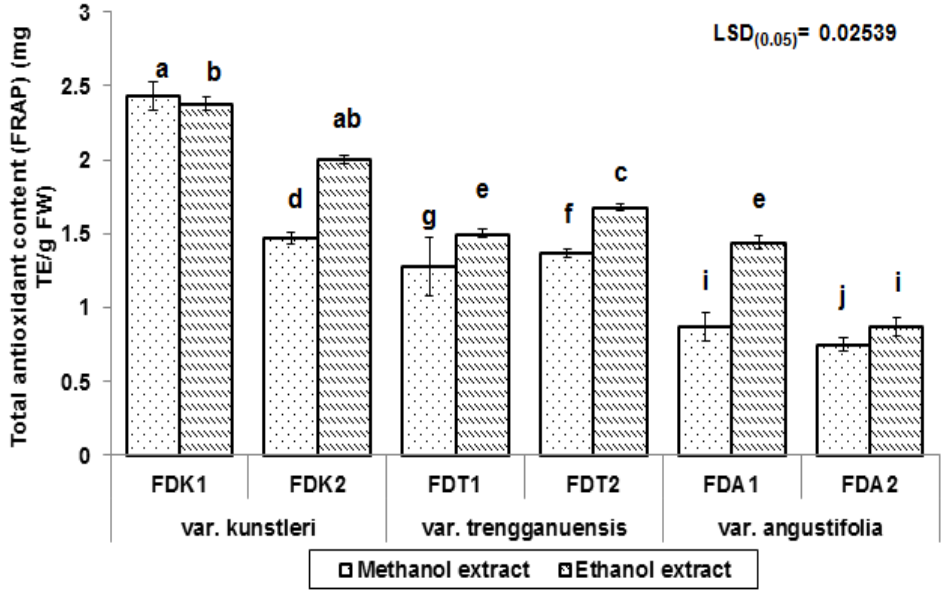

Figure 2: Total antioxidant content (FRAP method) of *Ficus deltoidea* varieties in methanol and ethanol extractions. Bar indicates the standard error of mean (n=3). Means with different letters are significantly different at $p<0.05$ from each other according to LSD post hoc analysis.

Wong *et al.* (2006) reported that DPPH assay and FRAP assay have a strong correlation between the mean values in all samples tested, which indicated that the extracts are capable of reducing DPPH radicals and ferric ions. Various researchers reported high total antioxidant content in different species of *Ficus* using different methods for analysis. Earlier study reported by Ao *et al.* (2008) revealed a high antioxidant activity from leaf extract of *Ficus microcarpa* compared to extracts of bark and fruit. Results from this study showed that plant extract from genus *Ficus* has high antioxidant activity, especially in the leaf of the plant. Study in 30 plant extracts disclosed that wood extract of *Quercus robur* measured using FRAP method exhibited the highest total antioxidant content of 15.92 mmol Fe^{2+}/g, while flower extract of *Matricaria recutita* contained the least with 0.12 mmol Fe^{2+}/g (99% difference between the highest and lowest total antioxidant content) (Dudonné *et al.*, 2009). In this study, the difference between the highest and lowest total antioxidant content in ethanol and methanol extracts of *F. deltoidea* was 63% and 69% respectively, which was lower compared to Dudonné *et al.* (2009). Results obtained from this study reveal that DPPH free radical scavenging assay and FRAP assay are both reliable, rapid, easy and accurate methods, which can be applied in monitoring the activity of numerous samples over a limited period of time (Klimczak *et al.*, 2007; Maisuthisakul *et al.*, 2008).

Total Polyphenol, Phenolic Acid and Flavonoid Content in Methanol and Ethanol Extracts

Figure 3 shows total polyphenol content in methanol and ethanol extracts of *F. deltoidea* varieties. Total polyphenol content in ethanol extracts was higher compared to that of methanol extracts in all varieties studied. Leaf extract of FDK1 contained the highest activity for both methanol and ethanol extracts with 1.12 and 1.13 mg GAE/g FW, thus not significantly different to each other. Leaf extracts of FDK2 and FDT2 contained the same amount of total polyphenol content in methanol extract with 0.61 mg GAE/g FW. The lowest activity of methanol extract can be found in leaf extract of FDA1 with 0.50 mg GAE/g FW. In ethanol extract, leaf extracts of FDK2 showed considerably high total polyphenol content followed by FDT1 and FDA1 with 0.90, 0.80 and 0.71 mg GAE/g FW, respectively. The lowest total polyphenol content for ethanol extracts was found in leaf extract of FDA2 with 0.56 mg GAE/g FW. The decreasing order of total polyphenol content in methanol and ethanol extracts by variety can be arranged as follows; *F. deltoidea* var. *kunstleri* > var. *trengganuensis* > var. *angustifolia.*

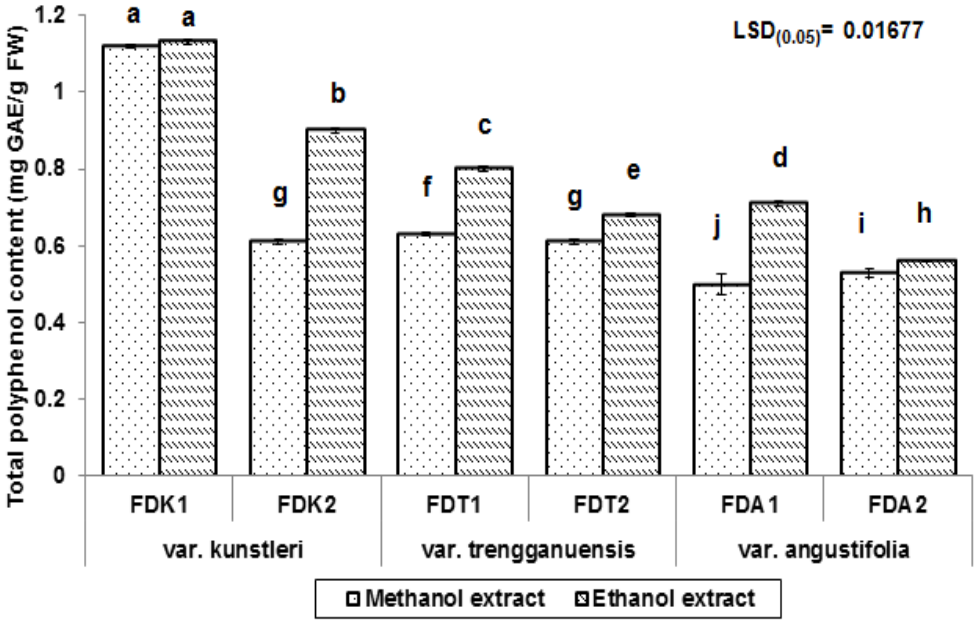

Figure 3: Total polyphenol content of *Ficus deltoidea* varieties in methanol and ethanol extractions. Bar indicates the standard error of mean (n=3). Means with different letters are significantly different at $p<0.05$ from each other according to LSD post hoc analysis.

A study using different solvents to study antioxidant capacity in *Prunus serotina* subspecies *capuli* showed that ethanol extract contained higher total antioxidant content as measured using percentage of inhibition, with 73.5% compared to that of methanol extract with 72.7%. The same observation was found in total polyphenol content, in which the ethanol extract exhibited a higher total polyphenol content with 1732 mg/100 g compared to the methanol extract with 1649 mg/100 g (Jimenez *et al.*, 2013). The findings indeed corroborated results obtained from our study where all the leaf extracts using ethanol extraction possessed a higher total polyphenol content compared to that of methanol extraction. However, the amount of total polyphenol may differ based on changes in parameters such as temperature, pH value and time of harvest.

Figure 4 shows total phenolic acid content in methanol and ethanol extracts of *F. deltoidea* varieties. The highest total phenolic acid content was found in leaf extract of FDK1 using methanol extract with 4.54 mg GAE/g FW. In ethanol extract, leaf extract of FDK2 showed the highest activity of total phenolic acid content with 4.00 mg GAE/g FW. Leaf extracts of FDK2 and FDT1 in methanol extract were not significantly different to each other with 2.54 and 2.55 mg GAE/g FW, respectively. The lowest total phenolic acid content of both extracts was found in leaf extract of FDA2 with 1.81 and 1.30 mg GAE/g FW, respectively. Other leaf extracts with ethanol showed a considerable amount of total phenolic acid content such as leaf extract of FDK1 with 3.90 mg GAE/g FW followed by leaf extracts of FDT1 and FDT2 with 3.00 and 2.80 mg GAE/g FW, respectively.

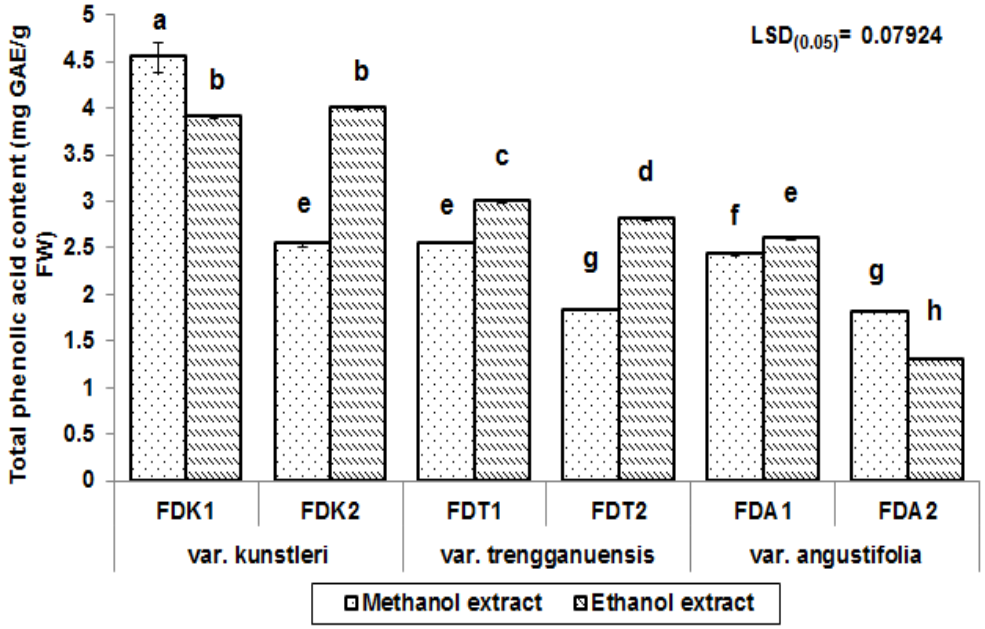

Figure 4: Total phenolic acid content of *Ficus deltoidea* varieties in methanol and ethanol extractions. Bar indicates the standard error of mean (n=3). Means with different letters are significantly different at $p<0.05$ from each other according to LSD post hoc analysis.

A study on the nutritive compounds in *Sorghum bicolor* (Guinea corn) revealed that methanol extract produced a higher total phenolic acid content of 1036 mg/mL compared to ethanol extract with only 847 mg/mL. However, other biochemical compounds such as total carotenoids, lycopene, β-carotene, chlorophyll a and chlorophyll b contents were found higher in ethanol extract compared to that of methanol extract (Abugri *et al.*, 2013). However, our findings disclosed that ethanol extracts exhibited higher total phenolic acid content in leaf extracts of *F. deltoidea* compared to that of methanol extracts. Previous study on total phenolic acid concentrations and antioxidant activity of purple-fleshed potatoes as affected by boiling treatment discovered that differences on solvents percentage could influence the production of total phenolic acid and total antioxidant contents (Burgos *et al.*, 2013). Meanwhile, a study conducted to investigate the activity in extracts of Colombian Amazonian plants known to contain medicinal usage showed that leaf extract of *Piper putumayoense* exhibited the highest total

phenolic acid with 22.20 mg GAE/g, with the lowest was detected in stem extract of *Iribachia alata* with 0.81 mg GAE/g (Lizcano *et al.*, 2010). Data presented by Lizcano *et al.* (2010) was higher compared to that of leaf extracts of *F. deltoidea* in methanol and ethanol extracts obtained from our study. The highest total phenolic acid for methanol extract was found in leaf extract of FDT1 with 4.54 mg GAE/g FW, while leaf extract of FDK2 contained the highest total phenolic acid using ethanol extract with 4.00 mg GAE/g FW.

From Figure 5, leaf extracts of *F. deltoidea* extracted using ethanol contained a higher total flavonoid content compared to that of methanol extraction. Leaf extract of FDK1 showed the highest activity of total flavonoid for both methanol and ethanol extracts with 5.83 and 6.81 mg RE/g FW, respectively. In methanol extract, most of the leaf extracts contained low amount of total flavonoid content such as the leaf extracts of FDT2, FDA1 and FDA2 (1.88, 1.04 and 0.67 mg RE/g FW). On the other hand, the total flavonoid content in ethanol extract displayed high activity in leaf extracts of FDK2, FDA1 and FDA2 (4.53, 4.44 and 3.85 mg RE/g FW). The order of total flavonoid in methanol extract by varieties is as follows; *F. deltoidea* var. *kunstleri* > var. *trengganuensis* > var. *angustifolia* while for ethanol extract; *F. deltoidea* var. *kunstleri* > var. *angustifolia* > var. *trengganuensis*.

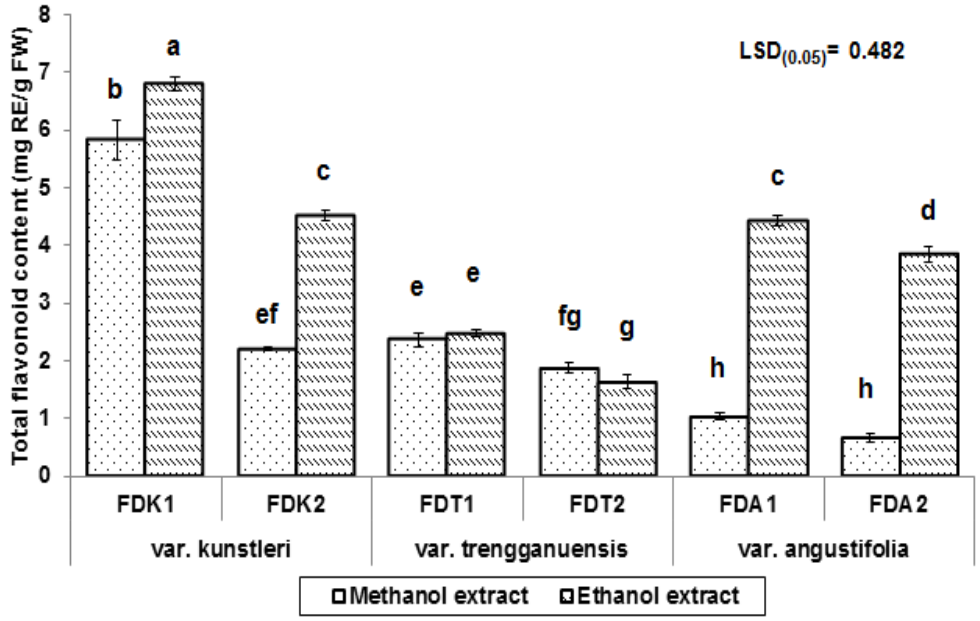

Figure 5: Total flavonoid content of *Ficus deltoidea* varieties in methanol and ethanol extractions. Bar indicates the standard error of mean (n=3). Means with different letters are significantly different at $p<0.05$ from each other according to LSD post hoc analysis.

A study on saffron stigma discovered that methanol extract produced higher total flavonoid and total phenolic contents with 5.8 mg/g DW and 6.5 mg/g DW respectively, while ethanol extract only produced 2.9 mg/g and 6.3 mg/g of total flavonoid and total phenolic contents (Karimi *et al.*, 2010). These findings were not in agreement with the results obtained in this study, where we revealed that ethanol exhibited higher bioactive compounds in most of the extracts studied. Other study on leaf extracts of *Cistus ladaniferus* postulated that methanolic extract contained a higher total flavonoid content compared to ethanolic extract with 64.33 and 61.40 mg rutin/g of extract (Amensour *et al.*, 2010), which is not in agreement with this current study where ethanolic extracts of *F. deltoidea* exhibited higher total flavonoid content compared to that of methanolic extract.

Flavonoid Compounds Separation of *Ficus deltoidea* Varieties

From Table 1, the production of rutin was the highest in leaf extract of *F. deltoidea* var. *kunstleri*, while the highest naringin was detected in leaf extract of *F. deltoidea* var. *trengganuensis*. The leaf extract of accession FDK1 contained 12.83 µg/g DW of rutin with 87% higher than that of detected in leaf extract of accession FDT1 which contained the lowest rutin production (1.65 µg/g DW). Leaf extract of accession FDK2 contained 8.08 µg/g DW

of rutin, followed by leaf extracts of FDA1, FDT2 and FDA2 with 5.97, 3.74 and 2.18 μg/g DW, respectively. However, naringin production was lower compared to that of rutin production in all of the leaf extracts studied. There was 76% difference between the highest rutin production and the highest naringin production. The highest production of naringin was found in leaf extracts of accession FDT2 with 3.04 μg/g DW, while the lowest was found in the same accession with 0.14 μg/g DW. Leaf extract of FDA2 contained 1.21 μg/g DW, followed by leaf extracts of accessions FDA1, FDK1 and FDK2 (0.92, 0.89 and 0.74 μg/g DW).

Table 1: Quantification of individual flavonoid in leaf extracts of *Ficus deltoidea* varieties using gradient ratio of 1 to 35% of acetonitrile to ultra-pure water at pH 2.5 detected at 365 nm for 30 min and 1.0 ml/minute flow rate.

Flavonoid content (μg/g DW)	var. *kunstleri*		var. *trengganuensis*		var. *angustifolia*	
	FDK1	FDK2	FDT1	FDT2	FDA1	FDA2
Rutin	12.83 ± 0.94^{a}	8.08 ± 0.78^{b}	1.65 ± 0.17^{fg}	3.74 ± 0.27^{d}	5.97 ± 0.39^{c}	2.18 ± 0.16^{ef}
Naringin	0.89 ± 0.07^{gh}	0.74 ± 0.07^{gh}	0.14 ± 0.04^{h}	3.04 ± 0.23^{de}	0.92 ± 0.10^{gh}	1.21 ± 0.13^{fgh}

Data were taken from triplicate experiments. Means that did not differ significantly at the 5% level of significance when compared with Tukey's HSD test are followed by the same superscript letters.

A study on kumquat (*Fortunella japonica* Swingle) juice showed that five flavonoid compounds namely acacetin 3,6-di-*C*-glucoside, vicenin-2, lucenin-2 4'-methyl ether, narirutin 4'-*O*-glucoside and apigenin 8-*C*-neohesperidoside were identified for the first time out of 13 flavonoids found, with the highest activity of the juice was flavonoid compound known as phloretin 3',5'-di C-glucoside (Barreca *et al*., 2011). Previous findings on *Ulmus davidiana* extracts discovered that out of three flavonoids ((–)-catechin, (–)-catechin-7-O-β-D-apio-furanoside and (–)-catechin-7-O-β-D-xylopyranoside) studied, only (–)-catechin compound had the capacity to chelate metal moderately, while the rest showed weak metal chelating ability (Jung *et al*., 2010). Meanwhile, a study on *Artemisia absinthium* revealed that artemisetin was the only flavonoid compound detected using LaChrom Elite HPLC system (Merck-Hitachi, Tokyo, Japan), an autosampler (L-2200), L-2100 quaternary pump, L-2300 column oven and l-2400 UV-detector with the mobile phase of 0.085% orthophosphoric acid in water and acetonitrile. Artemisetin was known to exhibit several uses and beneficial properties such as antiinflammatory, antitumor and antiproliferative activities (Aberham *et al*., 2010). From our study, the leaf extract of *F. deltoidea* var. *kunstleri*, accession FDK1 showed the highest production of rutin and consistently showed high total antioxidant content with other biochemical properties related to antioxidant studies.

CONCLUSIONS

As a conclusion, the ethanol extracts of *F. deltoidea* varieties produced higher total antioxidant content compared to the methanol extracts. It is also revealed that rutin and naringin were expressed in all of the intact plants, with rutin was more dominant than naringin. The leaf extract of FDK1 from *F. deltoidea* var. *kunstleri* continuously possessed high activity of flavonoid.

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